

BREEDING RESEARCH ON SOUTH AFRICAN PLANTS:

II. FERTILITY OF PROTEACEAE.

MET OPSOMMING IN AFRIKAANS

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ABSTRACT

The seed set in open-pollinated flowers of *Protea*, *Leucospermum* and *Serruria* spp. was investigated and found to be low. Controlled self-pollinations revealed self-incompatibility in *Protea* and some *Leucospermum* spp. but self-compatibility in other *Leucospermum* spp. and *Serruria florida*. Investigation of the microsporogenesis and *in vitro* pollen germinations proved the viability of the male gamete. Carefully screened seed of *Protea* spp. germinated satisfactorily in unsterilized and sterilized soil as well as in vermiculite. *Leucospermum* and *Serruria* germination did not reach the high level of that in *Protea*. It is assumed that in all three genera good seed germination may be obtained merely by an effective method of sorting the sound from the unsound seeds. The results are discussed with regard to the possible presence of germination inhibitors, the possible role of microbes in germination, the natural mode of pollination in the genera mentioned and the importance thereof for future plant breeding work.

INTRODUCTION

From a recent survey on research work done on the family Proteaceae Juss. (Vogts, 1960), it becomes evident that no previous investigations have been made to clarify the mode of pollination in this family, or to explain the very low fruit set which is generally experienced in some South African genera such as *Protea* L. and *Leucospermum* R. Br. A more detailed knowledge of the natural and other possible modes of pollination, the generation turnover and the sexual propagation rate is however imperative for a breeding programme on Proteaceae, initiated at the above Institute in 1957.

PROCEDURE

The material used in this experiment was collected in the natural habitat or obtained from the National Botanic Gardens, Kirstenbosch, Cape, from commercial sources and also from plants grown at the experimental farm at Groot Drakenstein, Cape.

Plants chosen for the pollination experiments were isolated by the time of flowering or the flower heads were bagged with glassine bags.

Three sowing media were used: horticultural vermiculite, sterilized and unsterilized soil mixtures. Sterilization was effected by a temperature of at least 180°F for half an hour in an autoclave or a soil-sterilizer. The expression "seed" will be used instead of the botanically correct "fruit" in this paper. All the seeds from these experiments were carefully screened and only those being plump and able to resist some pressure were sown (= sorted seed). So-called unsorted seeds, mentioned later, were sown as received from the different sources after having been checked for mechanical damage, insect infestations, disease and immaturity. The seeds were sown during March and April.

Cytological investigation of pollen mother cells was carried out after fixing in 1:3 acetic alcohol and staining with 0.25% aceto-carmin containing 1.5% of a 10% FeCl₃ solution. *In vitro* pollen germination tests took place in a drop of water containing 50 ppm boric acid plus 25% sucrose for *Leucospermum* and 25% sucrose for *Protea*.

RESULTS

(1) *Germination of seeds*

Sorted *Protea* seeds from open-pollinated and non-isolated flowers germinated satisfactorily in all three media mentioned. With respect to vermiculite it was found that seed germinated equally well in the dark or under daylight conditions. The germination percentages as observed in sterilized soil are given in Table 1. In this table germination figures for each individual species, having approximately the same germination ability, were grouped in the following classes: (A) more than 66%; (B) between 66% and 33%; and (C) below 33% germination.

TABLE I: GERMINATION OF *PROTEA*

Tabel I: ONTKIEMING BY PROTEA

	Number of seeds sown <i>Aantal saad gesaa</i>		Mean Germination % <i>Gem. Ontkiemings %</i>	
	Sorted <i>Gesorteer</i>	Unsorted <i>Ongesorteer</i>	Sorted <i>Gesorteer</i>	Unsorted <i>Ongesorteer</i>
Group A ¹ <i>Groep</i>	2,861	4,272	75.9 ± 7.7	11.2 ± 11.6
Group B ² <i>Groep</i>	1,754	2,328	55.4 ± 5.3	10.3 ± 6.5
Group C ³ <i>Groep</i>	261	3,009	21.3 ± 5.8	8.5 ± 8.2

- ¹ Mean germination : > 66%; includes: *P. compacta* hybr., *P. cynaroides*, *P. Gemiddelde ontkieming:* sluit in: incompta, *P. latifolia* hybr., *P. longiflora*, *P. longiflora* hybr., *P. neriifolia*, *P. neriifolia* hybr., *P. mundi*.
² Mean germination : > 33% < 66%; includes: *P. barbiger*, *P. compacta*, *P. Gemiddelde ontkieming:* sluit in: laticolor, *P. lanceolata*, *P. laurifolia*, *P. minor*, *P. pulchella*, *P. susannae*.
³ Mean germination : < 33%; includes: *P. mellifera*, *P. longifolia*.
Gemiddelde ontkieming: sluit in:

Unsorted seed germinated unsatisfactorily. The difference between the mean germination percentage of sorted and unsorted seeds is significant in groups A and B. ($P = 0.1\%$). The influence of the seed hairs on the germination shows a positive effect. Comparing the germination of seeds within the same species it was found that seeds with hairs gave 40.2% germination against 19.5% of the seeds with hairs rubbed off. This difference is significant ($P = 1\%$).

In *Leucospermum* and *Serruria florida* Knight only low germination was obtained, which, however, could be improved by a more effective seed-sorting procedure, as shown in Table 2. Some apparent discrepancies in the relation between the germination percentages of plump seed on the one hand

TABLE 2: GERMINATION OF LEUCOSPERMUM AND SERRURIA
Tabel 2: ONTKIEMING BY LEUCOSPERMUM EN SERRURIA

	No. of seed sown <i>Aantal saad gesaai</i>			Mean germination % <i>Gem. ontkiemings %</i>
	Plump <i>Dik</i>	Plump and light ¹ <i>Dik en lig¹</i>	Plump and heavy ¹ <i>Dik en swaar¹</i>	
<i>L. attenuatum</i> ..	905	—	—	12.5 ± 10.7
<i>L. bolusii</i>	2,184	—	—	22.6 ± 8.3
	—	201	—	29.8 ± 3.2
	—	—	156	49.3 ± 6.4
<i>L. muiirii</i>	144	—	—	12.5 ± 10.6
<i>L. nutans</i>	1,092	—	—	33.4 ± 11.7
<i>L. reflexum</i>	2,276	—	—	9.3 ± 5.1
	—	308	—	1.6 ± 1.2
	—	—	247	7.3 ± 3.8
<i>S. florida</i>	2,476	—	—	14.4 ± 12.9
	—	152	—	2.6 ± 1.2
	—	—	330	15.1 ± 8.2

¹ Light seeds float on the surface of water whereas heavy seeds sink.

¹ *Ligte saad drywe op die oppervlakte van water terwyl swaar saad afsak.*

and plump and heavy or plump and light seed on the other hand may be explained by the fact that experimentation with seed weight took place in a different season.

(2) *Seed set in open-pollinated flowers*

Seed set was found to be very low even in flower-heads collected from populations in the natural habitat. The data are given in Table 3, on number of flowers per flower-head and the number of plump seeds per

TABLE 3: FERTILITY IN OPEN-POLLINATED FLOWERS IN PROTEACEAE

Tabel 3:

VRUGBAARHEID VAN VRY-BESTUIFDE BLOMME IN PROTEACEAE

	Number of heads used <i>Aantal hoofde gebruik</i>	Mean number <i>Gem. aantal</i>		% Fruit % <i>Vrugte</i>
		Flowers/Head <i>Blomme/Hoof</i>	Plump Fruits/Head <i>Dik Vrugte/Hoof</i>	
<i>PROTEA</i>				
<i>barbigera</i>	18	429.7 ± 144.0	12.0 ± 7.8	2.8
<i>cedromontana</i>	13	101.9 ± 22.9	—	—
<i>compacta</i>	33	89.7 ± 18.0	15.1 ± 9.8	16.8
<i>compacta</i> <i>hybr.</i>	20	294.6 ± 76.2	9.8 ± 4.3	3.3
<i>incompta</i>	37	289.1 ± 49.9	10.1 ± 10.6	3.5
<i>lacticolor</i>	21	94.3 ± 28.7	4.9 ± 4.2	5.2
<i>lanceolata</i>	12	56.9 ± 8.5	6.5 ± 2.2	11.4
<i>latifolia</i>	37	144.2 ± 21.0	34.8 ± 29.4	24.1
<i>laurifolia</i>	29	364.0 ± 56.1	4.8 ± 6.0	1.3
<i>longiflora</i>	16	—	3.6 ± 3.2	—
<i>longiflora</i> <i>hybr.</i>	70	169.9 ± 27.6	10.9 ± 12.7	6.4
<i>longifolia</i>	42	256.1 ± 83.9	1.8 ± 2.5	0.7
<i>mellifera</i>	45	78.2 ± 19.4	55.2 ± 16.3	70.3
<i>minor</i>	12	249.4 ± 154.9	4.3 ± 2.3	1.2
<i>mundii</i>	18	95.9 ± 28.6	3.3 ± 2.7	3.4
<i>nerifolia</i>	35	297.1 ± 63.9	10.7 ± 13.4	3.6
<i>nerifolia</i> <i>hybr.</i>	48	338.4 ± 105.2	19.0 ± 16.1	5.6
<i>obtusifolia</i>	57	283.7 ± 65.0	3.5 ± 3.1	1.2
<i>pulchella</i>	32	149.3 ± 58.2	14.5 ± 12.8	9.7
<i>recondita</i>	7	47.4 ± 12.1	—	—
<i>scolymocephala</i>	14	79.5 ± 45.7	3.4 ± 2.6	4.3
<i>susannae</i>	30	161.5 ± 22.7	10.0 ± 8.3	6.2
<i>LEUCOSPERMUM</i>				
<i>attenuatum</i>	271	108.6 ± 5.8	1.3 ± 0.4	1.2
<i>bolusii</i>	549	157.1 ± 7.5	3.8 ± 3.0	2.4
<i>conocarpum</i>	17	103.6 ± 7.1	4.9 ± 6.3	4.7
<i>nutans</i>	167	147.7 ± 8.1	3.2 ± 3.0	2.2
<i>reflexum</i>	422	161.1 ± 13.3	3.7 ± 3.7	2.3
<i>SERRURIA</i>				
<i>florida</i>	176	—	13.1 ± 4.4	—

flower-head. They show that in the majority of *Protea* spp. investigated the mean percentage of flowers which developed plump seeds is less than ten, one exception being *P. mellifera*. This impression is, however, erroneous, since the low germination in this species indicates errors in separating sound and plump from plump but unsound seed. In *Leucospermum* the seed set is equally low, while in *Serruria florida* it is higher than in the other genera.

Observations were also made with regard to the duration of the juvenile stage of a species, i.e. of the time elapsed until a plant first flowered. Most of the species mentioned started flowering in the third or fourth season after sowing.

(3) Investigation of microsporogenesis

No irregularities in the meiotic cycle were found in *P. arborea* and *P. barbigera*. Pairing in pachytene and diakinesis was perfect, the mean number of chiasmata being 84 ± 0.007 per chromosome. *In vitro* pollen tests gave a maximum germination of 94% in *Leucospermum* spp. and 86% in *Protea* spp.

(4) Controlled pollinations

In this experiment 19 plants belonging to 12 different *Protea* spp. viz. *P. compacta* hybrid, *P. laticolor*, *P. lanceolata*, *P. latifolia*, *P. laurifolia*, *P. longiflora* hybrid, *P. longifolia*, *P. mellifera*, *P. mundii*, *P. neriifolia*, *P. neriifolia* hybrid, *P. obtusifolia* and *P. susannae* were used. Almost 200 flower-heads with approximately 40,000 individual flowers were bagged and half of them were hand-selfpollinated. None of the mentioned species produced viable seed following mere isolation of the heads by glassine bags or isolation plus hand-selfpollination. Also not one of a few flower-heads isolated by their flowering time gave viable seed.

In *Leucospermum* 15 flower-heads of each of the species *L. attenuatum*, *L. bolusii*, *L. nutans* and *L. reflexum* were isolated by bags and one-third of which were self-pollinated. In *L. nutans* no plump seed was formed; in *L. bolusii* a mean of 0.4 plump seeds per flower-head were produced but these failed to germinate. In *L. attenuatum* and *L. reflexum*, however, flowers produced viable seed at a rate of 3.8 and 4.4 per head respectively. More than 300 flower-heads of *L. attenuatum* isolated by their time of flowering also set viable seed. From these seeds plants of normal vigour originated.

Flowers of 220 flower-heads of *Serruria florida* set seed freely when isolated by either of the above methods. Also in this case the seeds produced normal growing plants.

DISCUSSION AND CONCLUSIONS

Originally, the above experiments were initiated with the object of gaining knowledge which was of importance to the breeding project on Proteaceae. However, the results obtained have a bearing on published opinions as well as frequently accepted beliefs.

1. Germination

Some differences of opinion exist regarding the measures necessary for the germination of proteaceous seed.

Werner (1951, page 3) advises "that the seeds are no doubt encouraged to germinate by the even moist conditions." Vogts (1958) holds a similar opinion in stating that seedbeds should be kept damp, but not wet. The same author (Vogts, 1960, page 303), however, says later that seeds "will not germinate in good soil even if kept damp" and advises that the seeds "must be watered . . . in such a way that they are washed through and the inhibiting factor leached out." Furthermore Vogts (1960) found that seeds "will not germinate in sterilized sand" and "that soil microbes also play a part".

As a consequence of our own experiments and observations we are inclined to ascribe the main cause for the unsatisfactory germination in Proteaceae to ineffective means of separating fertile from infertile seed. The highly significant difference between sorted and unsorted seed with regard to germination cannot be explained otherwise. Also, the fact that in *Leucospermum* and *Serruria*, sound-looking but light seed germinated so much more poorly than heavy seed shows that the main task in improving the germination of proteaceous seed is to devise effective seed sorting methods. All the other aspects seem to be of secondary importance. The difficulty of this task is shown by our own observations, and also by those of Jordaan (1945 a, b, 1949) who in *P. mellifera*, also mentions the identical appearance of sound and unsound seeds. In *Leucadendron* he found that only 20 to 50% of sound-looking fruits were actually fertile.

Considering the influence of microbes on the germination of proteaceous seeds (Vogts, 1960) it is necessary to stress that in our experiments all seeds were successfully germinated in a steam-sterilized soil mixture and *Protea* seeds in pure vermiculite as well. This result does not favour the theory that soil-microbes play a decisive part in the germination of seeds of proteaceous genera. In exploded vermiculite, the microbial life is likely to be extremely poor while sterilized soil requires considerably more time to develop a microbial population than is needed for the germination of *Protea* seeds. Moreover, seed germinated well in both sterilized as well as unsterilized soil.

The question whether germination inhibitors play a role, keeping the

seeds dormant if not sufficiently well diluted (Vogts, 1960) cannot be decided after the present observations. If such an inhibitor is assumed it can easily be inactivated or leached in most *Protea spp.* by merely keeping the seed-bed damp and giving it an occasional thorough soaking. Excessive watering is definitely not necessary. Since germination-inhibitors may be produced, destroyed or sensitized by light (Audus, 1953), it may also be mentioned that *Protea* seeds germinate under daylight conditions as well as in darkness. One can conclude therefore, that light is of no importance in the germination of these seeds. It also may be mentioned that a cold temperature treatment had no other than a retarding effect on germination. The situation is somewhat different, however, in the *Protea spp.* mentioned in group C in Table 1 and mainly in *Leucospermum* and *Serruria* (see Table 2). The germination period in these genera is usually longer than in *Protea* and the germination percentages obtained are lower than one would expect from sound seed. This fact could be explained by either of the following assumptions: that our seed-screening method was not as effective as in *Protea* and that therefore, many of the plump seeds were not actually fertile or that a germination inhibitor is present and that it is different from the one possibly present in *Protea* since it is not as easily washed out. Our opinion is that in all three genera good seed germination may be had merely by an effective way of sorting the sound from the unsound seed prior to sowing. In deciding this question further experimentation is required.

2. Seed set

As the present results show, seed set is relatively low both under natural conditions as well as under cultivation. A similar situation was reported previously by Jordaan (1945 a, b; 1949). He found that only 10—20% of the ovaries formed normal fruits in *P. mellifera*. He also reported that *Leucospermum conocarpum* sets up to 16% normal fruits while in *Leucadendron spp.* 0 to 80% fruit set was observed. One can assume that no true gametic sterility exists in the closely related members of the South African Proteaceae, since it has been demonstrated that the male gametes are completely viable. This is in agreement with De Vos (1943) who does not mention any meiotic irregularities during microsporogenesis in different genera of the Proteaceae. The development of the female gamete was investigated in *Leucadendron* and *Leucospermum* by Jordaan (1945 a, b) and no irregularities were reported. The cause of the low number of fertile seeds should therefore be sought in other directions, possibly in para-sterility (Brieger, 1930) or mechanical reasons such as deficiencies in the pollinator fauna.

3. Mode of Pollination

The question of pollinating agents is of importance when investigating the natural mode of pollination in *Protea*, *Leucospermum* and *Serruria*.

The pollinator fauna of the Proteaceae was investigated recently by Vogel (1954). He reviews the older literature on that subject which reported nectarinides as the main pollinating agents. This author, however, found that in *Protea*, *Leucospermum* and other genera of the family Proteaceae hymenopters also play a role. After studying the biology of the flowers he considers therefore the Proteaceae as ornithophilous as well as melittophilous and in rare cases as anemophilous. Considering nectarinides as well as hymenopters as pollinating agents is in agreement with our own observations and with those of several commercial growers of proteaceous plants. Vogts (1958) also mentions that *P. cynaroides* sets seed after pollination by bees. These pollinating agents as well as the protandry reported by Delpino (Knuth, 1909), Vogel (1954) and Vogts (1958) should ensure cross-pollination. Further proof that cross-pollination is the natural mode of pollination, at least in *Protea* and *Serruria*, lies in the great number of natural interspecific hybrids known. Hybrids are known, *inter alia*, of *P. barbiger*, *P. compacta*, *P. lacticolor*, *P. laurifolia*, *P. longiflora*, *P. mundii*, *P. neriifolia* and *P. susannae*.

It could nevertheless be demonstrated that *Serruria florida* and several *Leucospermum* spp. set seed after spontaneous and artificial self-pollination. This would mean that in disagreement with Delpino (i.e.) *Leucospermum* spp. are capable of self-pollination. Furthermore, it was demonstrated that they are self-fertile, that is, they produce a viable progeny after self-fertilization. Remarks in the literature (Vogts, 1958) indicate that there are self-fertile *Leucospermum* spp. other than those mentioned here, for instance *L. catherinae* and *L. album* which are described as fair to good seed producers under cultivation. *Serruria florida* is also self-fertile.

In the *Protea* spp. investigated, however, it was found that this genus is self-incompatible. No seed developed from isolated flowers nor from artificially self-pollinated ones, though in a few cases some plump seeds were found, which however, failed to germinate. Vogts (1958) mentions a good seed set in *P. barbiger* after artificial pollination without however stating whether following self or cross-pollination.

4. Importance of results for future plant breeding work

For the projected breeding programme on Proteaceae it is of practical importance that the self-incompatibility in *Protea* allows of cross-breeding without previous emasculation. But it also means that no breeding

methods can be employed where any form of self-pollination is utilized. In the self-fertile *Leucospermum* spp. and *Serruria florida*, however, the development of improved, true breeding strains can be had by applying breeding techniques, usually employed for breeding of self-pollinated plants. This makes it possible to attain any breeding aim quicker than in cross-pollinated, self-incompatible plants. Cross-breeding, of course, is complicated by the necessity for emasculation which in the flower-heads of Proteaceae presents a formidable problem. Breeding of genera of the Proteaceae is of a long-term nature since the generation turnover is low due to the long juvenile stage of seedlings.

OPSOMMING

TEELNAVORSING BY SUID AFRIKAANSE PLANTE: II. VRUGBAARHEID VAN PROTEACEAE

Bestuivingsproewe het getoon dat 12 *Protea* spp. en *Leucospermum nutans* self-onverenigbaar is; *L. reflexum*, *L. attenuatum* en *Serruria florida* is egter self-verenigbaar en self-vrugbaar. Waarnemings ten opsigte van aantal goeie saad per vry bestuifde blomhoof het getoon dat net 'n klein gedeelte van die blomme bevrug word. Hierdie groot persentasie onvrugbaarheid is egter nie deur gametiese steriliteit veroorsaak nie, want sitologiese ondersoek en *in vitro* stuifmeel-ontkiemingstoetse het geen abnormaliteit te voorskyn gebring nie.

Die groot persentasie dowwe saad is verantwoordelik vir die lae ontkiemingspersentasies, wat gewoonlik gevind word, want na sorgvuldige sortering van die saad kon 'n bevredigende ontkieming veral in *Protea* verkry word. Dit was die geval in ongesteriliseerde en gesteriliseerde grond sowel as in vermikuliet.

Die resultate word bespreek met betrekking tot ontkiemings-rems-towwe, moontlike invloed van mikrobies op die ontkieming, die natuurlike bestuivingswyse van die drie genoemde geslagte en toekomstige teelwerk.

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